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Author manuscript

Bioorg Med Chem Lett. Author manuscript; available in PMC 2021 June 27.

Published in final edited form as:

Bioorg Med Chem Lett. 2019 August 15; 29(16): 2286–2289. doi:10.1016/j.bmcl.2019.06.024.

Styryl quinazolinones and its ethynyl derivatives induce myeloid differentiation

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Abstract

The tumor suppressor transcription factor CCAAT enhancer-binding protein α (C/EBPα) expression is down-regulated in myeloid leukemias and enhancement of C/EBPα expression induces granulocytic differentiation in leukemic cells. Previously we reported that Styryl quinazolinones induce myeloid differentiation in HL-60 cells by upregulating C/EBPα expression. To identify more potent molecule that can induce leukemic cell differentiation we synthesized and evaluated new series of styryl quinazolinones, ethynyl styryl quinazolinones, styryl quinolinones and thienopyrimidinones. Thienopyrimidinones were found toxic and styryl quinolinones were

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2019.06.024.

found inactive. Ethynyl styryl quinazolinone **39** and styryl quinazolinone **5** were found active on par with the earlier reported analogues **1** and **2** suggesting that the 5-nitro furan-2-yl styryl quinazolinones find a real promise in leukemic cell differentiation. The improved potency of **5** suggested that further modifications in the 5-nitro furan-2-yl styryl quinazolinones can be at the phenyl substitution at the 3-position of the quinazolinone ring apart from the 5-position of the heteroaryl ring.

Keywords

Styryl quinazolinones; Myeloid differentiation; CCAAT/enhancer binding protein; Thienopyrimidinone; Apoptosis

Introduction

Long-term survival of acute myeloid leukemia (AML) patients is low and drug makers are developing novel targeted therapeutics to treat AML.[1] Hindrance in differentiation is a common observation in all AML subtypes and promise lies in the induction of myeloid differentiation. At present, all *trans* retinoic acid (ATRA) is administered as the first line therapy to induce differentiation in patients with acute premyelocytic leukemia (APL).[2] Though ATRA treatment induces remission and constitutes a cure in nearly 70% of APL patients, it has no effect on other myeloid leukemias.[3]

Our group has studied the role of CCAAT enhancer-binding protein α (C/EBPα),[4] transcription factor essential for differentiation of cells in liver, lung, adipose tissues, and bone marrow and is required for granulocytic or monocytic differentiation. We proposed that increased C/EBPα expression and/or activity in AML can lead to myeloid differentiation and demonstrated that styryl quinazolinone analogue (**1**), induces C/EBPα activity which in turn enhances differentiation and leads to growth arrest and apoptosis of leukemic cells.[5] We further explored a series of styryl quinazolinones and identified **2** as potent C/EBPα inducer. [6] Among various heteroaryls in development as drugs quinazolinone[7] also finds significance.

Styryl quinazolinones are well studied for applications as anti-bacterials[8] and as anticancer drugs.[9] The interesting aspect of styryl quinazolinones is that they were explored as Heat shock protein 90 (HSP90) inhibitors,[10] tubulin polymerization inhibitors,[11,12] RAD51 inhibitors,[13,14] and also cause shortening of telomeres.[15] From our high throughput screen[5] and subsequent development,[6] we found that styryl quinazolinones induce C/EBPα expression in HL-60 cells and there by induce myeloid differentiation. We hypothesized that there may be connectivity between all these protein targets and styryl quinazolinones. We were driven to postulate the exact mechanism and pathway of the drug action and hence we screened a series of structurally variant styryl quinazolinones such as styryl quinazolinones, ethynyl styryl quinazolinones, thienopyrimidinones, styryl quinolinones to see same phenotypic myeloid differentiation. Herein we present our observation of the C/EBPα expression levels and subsequent myeloid differentiation capacity of various distinct styryl quinazolinones.

Styryl quinazolinones and thienopyrimidinones were synthesized[16] according to the reported synthetic protocols.[15] Briefly, 5-substituted benzoxazinone derivative (ii) was obtained from corresponding anthranilic acid (i) upon cyclisation using acetic anhydride. 5-Substituted benzoxazinone (ii) was treated with respective aniline under reflux to yield quinazolinone derivative (iii). Finally styryl derivatives 1 to 10 were obtained from the respective intermediate (iii) by heating with particular aldehydes in acetic acid (Scheme 1).

Thienopyrimidinones were synthesized directly from the corresponding derivative (v) upon heating with 5-nitro-furan-2-aldehyde in acetic acid solvent (Scheme 2). Ethynyl styryl quinazolinones and styryl quinolinones were procured from the earlier synthesis.[15]

All the 49 styryl quinazolinone derivatives (10 styryl quinazolinones, 5 thienopyrimidinones (Table 1) 24 ethynyl phenyl substituted styryl quinazolinones (Table S1) and 10 Styryl Quinolinones (Table S2)) were screened using wright-giemsa staining and NBT reduction assay at 10 μM concentration similar to control.[5] From the initial differentiation and apoptosis assay[5] we found that among the styryl quinazolinones (**1–10**) screened derivatives **1**,[5] **2**,[6] **5**, and **6** showed significant differentiation of HL-60 cells (Table 1). All thienopyrimidinones showed significant toxicity and minor differentiation was observed in the case of **11** (at 1 μM), **12** (at 3 μM), **13** (at 3 μM), and **14** (at 3 μM). At higher concentrations (more than 3 μM), all the thienopyrimidinones exhibited toxicity. Among the ethynyl styryl quinazolinones (Table S1), only **19** and **39** found to differentiate the HL-60 cells. Also all the quinolinones (Table S2) were found inactive towards differentiation or apoptosis. When we measured the increase in CD11b expression levels (Fig. 1) with these leads (**5**, **6** and **39**) along with the controls (**ATRA**, **1**, **2**) we found that only compound **5** exhibited 89% increase of CD11b expression at 10 μM concentration compared to **ATRA** showing 96% increase at 1 μM concentration. Compound 6 showed 59% increase at 10 μM concentration.

Further we examined gene expression levels of C/EBPα and its downstream target C/EBPε, which have important role in terminal granulocyte differentiation and maturation. When HL-60 cells were treated with **ATRA**, **1**, **2**, **5**, and 6 mRNA expression levels of *CEBPA* were increased in a time-dependent manner. Though compound **11** and **12** increased the *CEBPA* levels (Table 2) they did not increase CD11b levels which is measure of granulocytic differentiation of HL-60 cells. Increase in *CEBPE* levels was observed in the case of **1**, **2**, **5** and **6**.

Initially we observed a strong correlation between various oncological targets and small molecule styryl quinazolinone (Fig. 2). This inspired us to explore various distinct and analogous derivatives of styryl quinazolinones (**1–49**) to ascertain their activity towards inducing myeloid differentiation and C/EBPα expression. From the preliminary screen of all the compounds, we found that styryl quinolinones were inactive and completely lost activity due to the change in the heteroaryl ring by removal of one nitrogen. The toxicity of thienopyrimidinones **11** and **12** can be due to the replacement of the adjacent phenyl ring by the thiophene ring. Further this significant structural change might be the cause for the increase in *CEBPA* gene expression levels without significant increase in the CD11b expression levels. Among 24 ethynyl styryl quinazolinones only **19** and **39** showed

differentiation of HL-60 cells. This implies that the ethynyl substitution had little or no effect on the differentiation of the leukemic HL-60 cells. Only compound **39** showed minimal increase in CD11b expression, *CEBPA* and *CEBPE* gene expression levels.

This may due to preservation of structure other than ethynyl phenyl part and also due to increase in hydrophobicity at quinazolinone 3-position. Among the styryl quinazolinones **1–10**, all the derivatives with phenyl substitution at the styryl part were inactive except **1**, which is with the dihydroxy phenyl group. Compound **10** might have lost activity due to change in the position of methoxy to the *para* position from the original *ortho* position. Among the derivatives **2**, **5**, **6**, **7** all are with nitro furan substitution replacing phenyl ring of styryl substitution. All these derivatives contributed to differentiation of HL-60 cells except **7**, which induced only a differentiation and no apoptosis. This activity might be due to the slightly acidic carboxyl group at the phenyl ring R2, *meta* position. Compounds **2**, **5**, **6** with minor changes at the phenyl ring R2 and at quinazolinone 5-position were found to enhance differentiation and subsequent apoptosis to a greater level compared to all other derivatives except **ATRA**. **2**, **5**, and **6** enhanced *CEBPA* and *CEBPE* gene expression levels significantly compared to other derivatives. The 89% increase in CD11b expression level due to 7 μM **5** treatment was nearly similar to ATRA treatment and marked highest level of differentiation from the current set of styryl quinazolinones screened.

In conclusion, we found that distinct variations in the structure of styryl quinazolinones made the derivatives completely inactive towards inducing myeloid differentiation. The thieno derivatives led to toxicity. Analogous derivatives were found to retain the differentiation potential and compound **5** was found to be the potent derivative for inducing myeloid differentiation in HL-60 cells. Exploring derivatives with further changes at the 5-position of the quinazolinone ring and elucidating the exact mechanism of action of these molecules will be our future interest.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research is supported by the Singapore Ministry of Health's National Medical Research Council under its Singapore Translational Research (STaR) Investigator Award, and by the National Research Foundation Singapore and the Singapore Ministry of Education under its Research Centres of Excellence initiative. R.S. (Radhakrishnan Sridhar), is supported by Cancer Science Institute of Singapore. S.S.K is supported by National Institution of Health (R21CA178301 and R01CA169259), American Cancer Society (RSG-13–047), and Harvard Stem Cell Institute Blood Program (DP-0110–12-00). R.S. thanks CSIR-HRDG for the award of CSIR-SRAship (13(8906-A)/2017-pool) and also acknowledges CSIR, New Delhi, for financial support under the 12th Five Year plan project "Affordable Cancer Therapeutics (ACT)" (CSC0301). D.G.T is supported by the National Institution of Health (R35CA197697 and P01HL131477). Authors thank Dr. Brian W. Dymock and Prof. Go Mei Lin for their valuable comments and helpful discussions during the course of this work and manuscript preparation.

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16. Representative procedure for synthesis of styryl quinazolinones: Reaction of 2-methyl-3-arylquinazolin-4(3*H*)-one (**iii**, l mmol) or thienopyrimidinones (**v**, 1 mmol) with substituted benzaldehyde or furan aldehyde (l mmol) in acetic acid under reflux conditions for 4 h. Then the reaction mixture was quenched with sodium bicarbonate and extracted with ethyl acetate (4×25 mL). The concentrate was purified by column chromatography employing EtOAc/Hexane as an eluent to afford corresponding styryl quinazolinone.

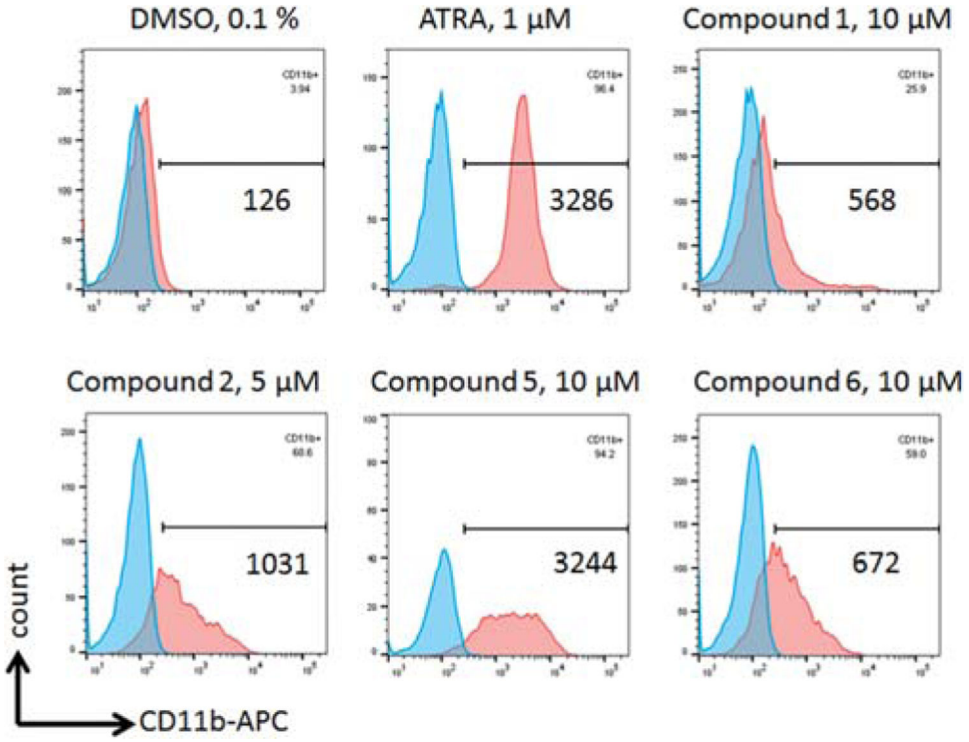


Fig. 1.

HL-60 treated with drugs for 7 days Anti human/mouse CD11b antibody – APC, Rat IgG2b.

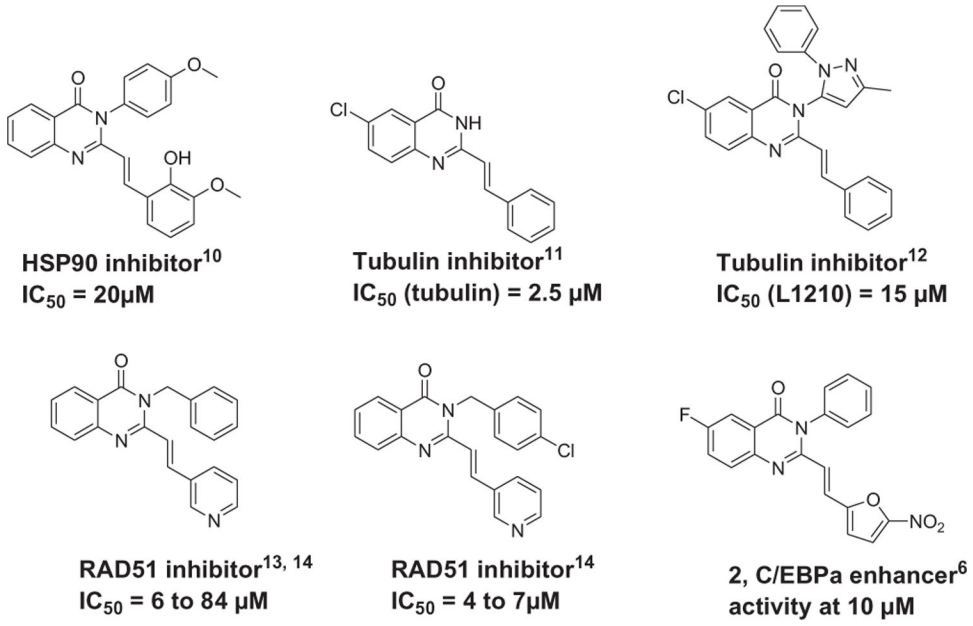
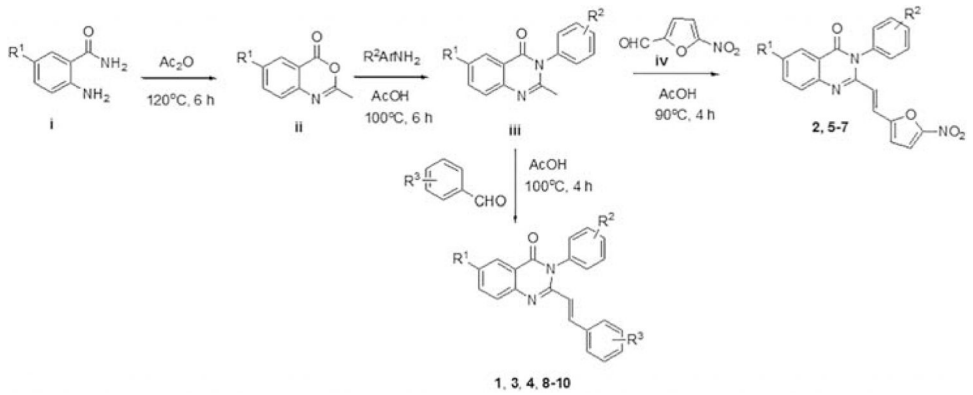
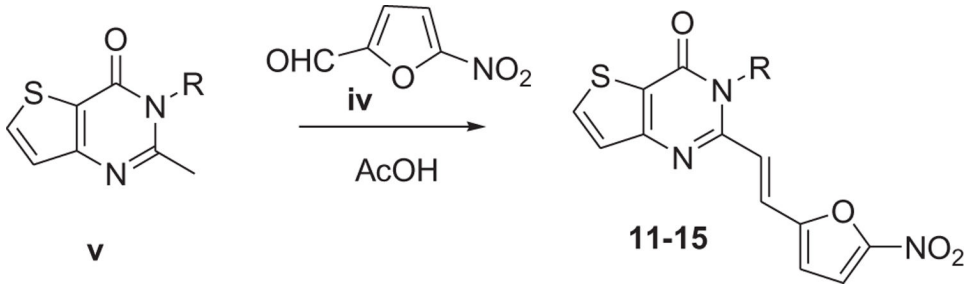


Fig. 2.
Interactions of Styryl quinazolinones with various cancer targets.



Scheme 1.

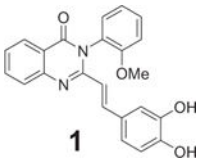
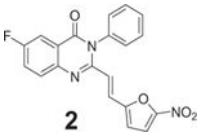
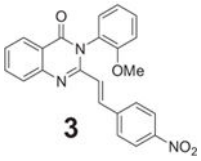
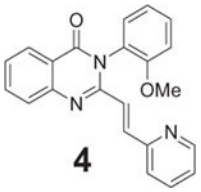
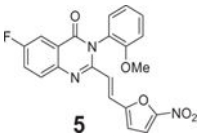
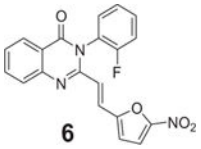
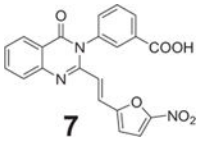
Representative synthetic route for styryl quinazolinones.

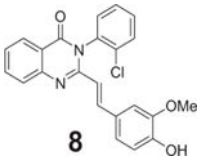
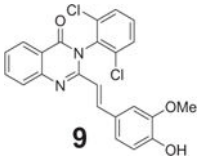
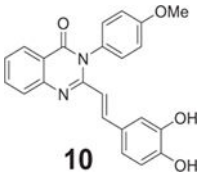
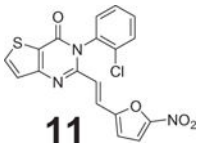
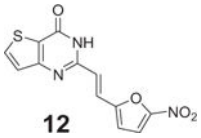
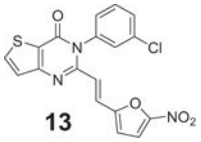
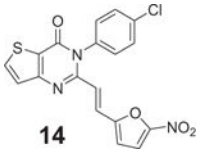
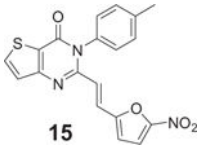


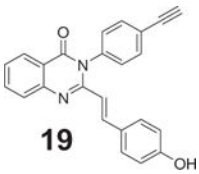
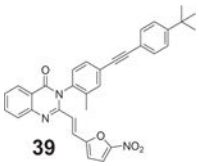
Scheme 2.
Representative synthetic procedure for styryl thienopyrimidinones.

Table 1

Synthesized and active styryl quinazolinones in HL-60 cell differentiation.

Structure	Concentration	Apoptosis	WG	NBT
DMSO[a]	0.1%	–	–	–
ATRA[b]	1 μM	+++	+++	+++
 1	10 μM	+	+	+
 2	3 μM	++	++	++
2  3	10 μM	–	–	_[c]
 4	10 μM	–	–	_[c]
 5	10 μM 3 μM	+++ ++	++ +	++ +
 6	10 μM 3 μM	+++ –	++ –	++ –
 7	10 μM	–	+	+

Structure	Concentration	Apoptosis	WG	NBT
 8	10 μM	−	−	−
 9	10 μM	+	−	−
 10	10 μM	−	−	−
 11	10 μM	+	−	+
 12	10 μM	+	−	+
 13	10 μM	+	−	±
 14	10 μM	+	−	±
 15	10 μM	+	−	−

Structure	Concentration	Apoptosis	WG	NBT
 19	10 μM	+	+	+
 39	10 μM	+	+	+

[a] DMSO used as control.

[b] ATRA used as positive control.

[c] Compounds **3** and **4** are purchased from the vendor and not synthesized.

Table 2

Synthesized active styryl quinazolinones in CD11b and ↑CEBPA gene expression levels.

Structure	↑ In CD11b in comparison with DMSO	↑ Gene expression of *CEBPA* (fold increase)	↑ Gene expression of *CEBPE* (fold increase)
DMSO	3.94	No change	No change
ATRA	96.4% at 1 μM	3 at 1 μM	ND
1	51.2% at 10 μM	1.5 at 10 μM	2.1 at 10 μM
2	42.2 at 3 μM	2.5 at 3 μM	10.3 at 3 μM
5	94.2%	1.6 at 7 μM	2.6 at 7 μM
	ND		
6	59.0%	1.8 at 10 μM	6.0 at 10 μM
	ND		

ND – denotes Not Determined.